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The Influence of Geomagnetism on the Maze Behavior of Dugesia Tigrina

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THE INFLUENCE OF GEOMAGNETISM ON THE MAZE BEHAVIOR OF DUGESIA TIGRINA

A Thesis
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the Graduate Faculty
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In Partial Fulfillment
of the Requirements for the Degree
Master of Science

by
Judith Kellman Miller
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APPROVED FOR THE GRADUATE FACULTY

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Judith K. Miller

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INTRODUCTION

The ability to learn is perhaps the most impressive of all the behavioral characteristics of living organisms. From the simplest forms of innate behavior to the most complex reasonings of man, we become aware of the many kinds of learning, all of which seem to be characterized by a lasting change in the behavior of the organism. Of the many classic definitions used to describe the abstract term, learning, perhaps that of Thorpe (1956) is the most satisfactory. He describes learned behavior as "the organisation of behaviour as the result of individual experience." It is the fact that this definition is so universal in its application and free of present-day theories of how the phenomenon is brought about, that makes it an excellent one. A newer definition of learning, put forth by McConnell (1964), is an example of one of the directions which contemporary research on learning is taking. McConnell proposes that we re-define learning as being "the end product of any set of events which causes a (someday hopefully specifiable) change in one or more (RNA?) molecules in an organism's cell(s). Whatever causes the chemical change also causes learning...... At last the hypothetical construct 'learning' could be given a meaning anchored in fact rather than in the never-never jargon of intervening variability."

In his search to understand learning, man will be satisfied when he understands not only how animals store information, but the nature of the information stored and the role of the nervous system in the entire process. One area in the research on learning has generated much interest and controversy in the past several years. This involves all the studies investigating learning and associated phenomena in the lower animals. The ability to learn has been ascribed to an enormously wide variety of organisms, including even those unicellular ones which possess no nervous system. It is because of our desire to discover where, on the phylogenetic scale, the capacity to learn emerges, that studies concerned with the alleged learning abilities of primitive organisms have generated such interest. We must know if learning is a basic property of all animals, if it depends on the development of a nervous system, what properties of the system are necessary, and the answers to the many other questions which have and will be proposed. It seems that the greatest problem in studying the behavior of the invertebrates stems from a lack of knowledge of these simpler forms in general. Much future experimentation will have to be carried out before we understand enough about the organisms themselves to avoid the experimental artifacts, errors in interpretation, and widespread inconsistencies which are so prevalent in the literature today.

The present study is concerned with investigating whether planarian flatworms are capable of learning in an instrumental conditioning paradigm, and whether or not this ability to learn is affected by orientation in the geomagnetic field. It is of interest to discover if an organism's orientation in the earth's magnetic field during training has any noticeable influence on its ability to learn a simple two-choice maze situation. Since the direction of the geomagnetic field has been found to have a definite effect on spontaneous orientation reactions of planarians, it seems likely that this factor may also affect the maze behavior of these organisms. Because this study involves experiments dealing with both learning and the effects of magnetism on planarians, the following review of the literature will attempt a brief summary of the research which has been reported in both of these areas.

REVIEW OF LITERATURE

I. Learning

Learning in a wide variety of lower forms has been reported for the last sixty years. Smith (1908) observed an increase in the facility of turning of paramecia placed in constricting capillary tubes. He designated this increased efficiency of an already present reaction as learning. Soest (1937), Gelber (1952), and others have reported attempts at classical and instrumental conditioning of paramecia. Ross (1964) reports the behavior of various sessile coelenterates in relation to some conditioning experiments. It is the studies on the flatworm that have stimulated the greatest amount of modern interest in the learning capacities of lower forms. Thompson and McConnell's (1955) demonstration of classical conditioning in planarians, and the tremendous amount of controversy concerning its validity which has followed, served to generate this recent interest.

Early Studies Concerning Learning in Flatworms

Walter's (1908) demonstration of habituation is the first study of a behavioral modification which may be related to learning in planaria. He observed that a slight rotation of the aquarium produced a halt in the gliding action of subjects, and this halting diminished if the rotation was repeated at one-second intervals. Van Oye (1920) is given credit for the first attempt to study a more complex behavioral change than habituation. His study, an early example of instrumental conditioning, involved training planarians to crawl on a tiny wire in order to reach food. Hovey (1929) conducted another early experiment in which he attempted to demonstrate that a marine flatworm could be trained to reverse an innate taxis. Leptoplana sp., usually quiescent in darkness and active when exposed to light, were observed to remain immobile in the presence of light following repeated touches on the snout. No further behavioral studies on flatworms were reported until 1937 when Soest and Dilk observed what may be regarded as avoidance learning. Soest (1937) conditioned Stenostomum sp. to remain in either the illuminated or darkened side of a circular bowl by shocking subjects as they crossed into the other half. Dilk (1937) had some success in similar experiments on planarians. Because Soest and Dilk failed to employ controls for sensitization, it is possible that the shock sensitized the animals only to the change in stimulation, and that no avoidance learning actually took place.

Recent Studies Concerning Learning in Planarians

Following this early work, studies in this area were largely neglected until 1955. As mentioned previously, modern interest in planarian learning was generated with Thompson and McConnell' s (1955) first controlled demonstration of classical conditioning in this phylum. Since then, the work on planarians has been concentrated essentially in three general areas: (1) additional classical conditioning experiments and discussions, (2) research concerning the locus of learning with its biochemical implications, and (3) studies investigating instrumental conditioning. The following includes the important findings which have been reported in each of these areas.

Classical Conditioning. Thompson and McConnell (1955) demonstrated in planarians an increase in response to light following a "training" session consisting of exposures to paired light (CS) and shock (UCS). Their data suggest that this increase in response to light is evidence that classical conditioning has occurred. Each training trial, consisting of three seconds of light accompanied by one second of shock during the final second, was administered as subjects crawled in a small, water-filled trough. In the experimental group, the frequency of responses to light (prior to shock) showed a significant increase in both body contractions and cephalic turns. Controls exposed to repeated shocks, repeated lights, or neither, all showed a slight decrease in response. Some strikingly different results were obtained by Cummings and Moreland

(1959) in a similar experiment using vibration (CS) and shock (UCS). Their controls, which were exposed to vibration only, showed the same rise in responsiveness as did the experimental subjects. Baxter and Kimmel (1963), in a three-part experiment, compared paired presentation with alternation of light and shock, two different shock intensities, and two different light intensities. They found that the groups exposed to paired stimuli were superior during acquisition training to the groups receiving alternate light and shock presentations.

The tremendous amount of controversy which has arisen seems to be due to a lack of agreement on a good definition of classical conditioning. Proponents of Thompson and McConnell's findings feel that the observed increased responsiveness to the CS (stimulus which normally produces no response, or a response different from that evoked by the UCS) clearly indicates that classical conditioning has occurred. Opponents argue that the results of these experiments are clear-cut examples of pseudo-conditioning. Pseudo-conditioning is a term which refers to a behavioral response of an organism brought about by factors other than the paired stimuli (CS and UCS) being investigated. These unlearned modifications in behavior are produced simply by barraging the nervous system of an animal with nonspecific stimuli. When findings reported as classical conditioning are actually based upon confounding, special pleading, ignoring certain aspects of data, ignoring previous research, or methodological errors, these findings are then cited as examples of pseudo-conditioning.

Jensen (1964) is one who feels there is little justification for the view that planaria can be classically conditioned. He bases his opinion on an observation by Pearl (1903), who noted that repeated strong stimulation decreases the number of positive reactions and increases the number of negative reactions given to any particular stimulus. Therefore, the results obtained by Thompson and McConnell (1955) and by Baxter and Kimmel (1963) can be explained as alternation in response to light by strong stimulation with shock. Studies demonstrating the importance of CS and UCS intensities (Baxter and Kimmel, 1963), the absence of differences between groups during extinction (Baxter and Kimmel, 1963; James and Halas, 1964), and the fact that light and shock both tend to produce similar behavior (Halas, James, and Stone, 1961; Halas, James, and Knutson, 1962; Hullett and Homzie, 1966) also support the pseudo-conditioning hypothesis.

Jacobson (1963) rejects the pseudo-conditioning interpretation because the evidence is based largely on the absence of differences between groups during extinction. He points out that in other studies (Corning and

John, 1961; Barnes and Katzung, 1963; Griffard, 1963) revealing significant differences in extinction data, the prior level of conditioning seems to have a direct effect on the type of extinction data obtained. Jacobson prefers Hilgard and Marquis' (1940) definition of classical conditioning which states that response increment is a "function of the repetition of conditioned and unconditioned stimuli in precise relationship." By this standard definition, all the previous studies which demonstrate differences in acquisition behavior between groups are examples of classical conditioning.

Some experimenters have reported little success in the classical conditioning of planarians. Barnes and Katzung (1963) have found that conditioning depends on whether the shock is delivered cathodally or anodally; Jacobson and Jacobson (1963) have found species to be an important factor; and Van Deventer and Ratner (1964) have disclosed the importance of such variables as temperature, shape of trough, and size of planarian.

Search for a Locus of Learning. The search to discover a locus of learning and the biochemical aspects of memory has received as much interest and skepticism as the studies on classical conditioning. The basic findings are as follows: (a) If classically conditioned worms are transected, the regenerates from the two halves show significant and equal savings on retest (McConnell, Jacobson, and Kimble, 1959). (b) If classically conditioned worms are fed to untrained cannibals, the latter manifest a higher response level to the CS than do cannibals fed untrained worms (McConnell, 1962). Westerman (1963) investigated regeneration and cannibalism in connection with his habituation experiments and his data seem to validate the findings reported earlier for classical conditioning. The necessity of adequate control measures in studies of this type can be seen if we mention the results obtained by Hartry, Morton, and Keith-Lee (1964). They found that control groups used as "food", which were handled but not trained, produced as much transfer as trained "food". The hypothesis that RNA is involved in this transfer has been receiving both support (Fried and Horowitz, 1964; Zelman et al., 1963; Corning and John, 1961) and skepticism (Dingman and Spron, 1964). Much additional investigation is needed in attacking the basis of "memory" in planarians. If these organisms do possess the ability to store a behavioral modification and pass this on to asexual progeny, extensive efforts to discover the mechanisms involved are certainly warranted.

Instrumental Conditioning. Jacobson (1963) reports that recent interest in instrumental learning in planarians was sparked by Ernhart and Sherrick's (1959) report of establishment of a simple maze habit. Instrumental conditioning differs from classical conditioning in that the animal is trained to do something in order to get or avoid something. The animal's response determines whether it is reinforced by punishment or reward. Planarians were trained to a criterion of three consecutive errorless trials in a water-filled T-maze in which the goal box was darkened. These were cut in half, allowed to regenerate, and both halves were again trained to criterion. Significant and equal savings were found in both regenerated heads and tails. Because the normal response of dark-adapted planarians is away from light (Pearl, 1903; Taliaferro, 1920), these findings may be due to sensitization rather than instrumental conditioning.

Best and Rubinstein (1962) have reported maze learning, where some subjects were trained to choose the lighted arm of the maze, thus eliminating this sensitization factor. Planaria were trained in a simple Y-maze with removal of water as the motivation to "run" the maze, restoration of water as the reinforcement, and light and darkness as the cues. The initial phase of enhanced preference for the reinforced alternative is cited as proof that instrumental learning had occurred. An abrupt decline in performance following the "learning" was an unexpected finding. There has been little criticism of this study other than that put forth by Jensen (1964), who questions the periods of rejection of the reinforced alternative. He cites Pearl's (1903) observation that light itself could produce turning toward or away from the light, depending upon other factors which influence whether the positive or negative reaction is given.

Jensen (1964) also quotes Pearl (1903) in his critical analysis of Lee's (1963) operant conditioning paradigm. Lee trained planarians housed in small lucite wells to intercept a small beam of light, with fifteen minutes of darkness as the reinforcement. Interception of the light beam by control subjects was ineffectual. Jensen and also Halas (1963) feel that the observed differences between experimentals and controls here is due to mechanisms other than operant conditioning. Planaria tend to move when the light is on and stop when it is off. Since the light goes off when the experimental animal intercepts the detector beam, this subject is stopped in a closer proximity to the beam than the control and hence is more likely to be in a position to trigger it again. The fact that the light beam is surrounded by a slight shadow is also suggested as having an influence on the results obtained by Lee. Best (1964) answers this criticism with alternative explanations.

A more recent study of instrumental conditioning (Humphries and McConnell, 1964) yielded results similar to those of Best and Rubinstein (1962). In a continuous Y-maze, subjects showed a marked increase in choice of a non-punished alternative, followed by a decline to the initial level.

The evidence supporting instrumental conditioning in planarians, like that of classical conditioning, seems impressive; however much further investigation is necessary before we can unequivocably accept these results. The approach here, which seeks to fit data to pre-established hypotheses, could be a factor causing the methodological errors and misinterpretations which are so evident. Also, the lack of knowledge of the planarian sensory apparatus and physiology and anatomy of the nervous system seriously hamper the choice of stimuli to be used in research.

II. Magnetic Effects

The biological effects of magnetic fields can be classified into three basic categories depending upon the factor causing each. One result of exposure to a static magnetic field is an interruption of the normal functioning of an organism. Such physical effects have been reported as retardation of growth (Barnothy, 1963), rejection of transplanted tumors (Barnothy, 1964), plant growth responses (Audus, 1960), and retardation of wound healing and tissue regeneration (Gross and Smith, 1961). Secondly, magnetic fields may have a stabilizing or labilizing effect upon the genetic code. It is the resulting changes in hydrogen bonds between the complementary nucleotide bases in the DNA molecule which seem to be involved in such phenomena as retardation of aging (Barnothy, 1960) and pathological changes in the adrenal (Sumegi, Barnothy, and Barnothy, 1964). A third group of effects appears to be based on an organism's oriented response to a type of sensory organ. This organ, which allows the specimen to sense fields of the order of the geomagnetic field, has probably been developing for millions of years. Rather than a type of compass-needle, this sensory device seems to be an extremely sensitive detector of currents. It is this sensory effect that is responsible for the orientation capacities of some animals, and will be discussed in the following review.

Recently much research has been focused upon two phenomena which persist in living organisms, and yet seem inexplicable in conventional physiological terms. These phenomena are the biological senses of time and space. The first includes the mechanism for timing well-known daily, tidal, monthly, and annual periodisms. The second involves the "map sense" or capacity to localize position in space. Because organisms are sensitive to subtle geophysical factors pervading the controlled laboratory conditions, there is general agreement that they are affected by both intrinsic and extrinsic regulating devices. Geomagnetism has been investigated as a possible extrinsic factor involved in organismic adaptation to the physical environment because of two of its qualities. First, time-intensity variations of some aspects of terrestrial magnetism appear to possess periods reflecting the natural atmospheric rhythmic changes. Second, since magnetism is a vector force, it could very possibly provide information important in the spatial orientation of organisms.

The effects of very weak magnetic field have been reported for a variety of organisms ranging from the unicellular *Paramecium* (Brown, 1962a), through *Volvox* (Palmer, 1963a; 1963b), *Dugesia* (Brown, 1962a), mud snails (Brown, Brett, and Webb, 1959; Brown, Brett, Bennett, and Barnwell, 1960; Brown, Webb, and Brett, 1960; Brown, Webb, and Barnwell, 1964), termites (Becker, 1963a), and Diptera (Becker, 1963b), to birds (Eldarov and Kholodov, 1964).

The spontaneous orientation reactions of animals provide a relatively simple and sensitive means for measuring biological response to weak magnetic fields. The majority of experiments of this type have concerned quantifying such reactions in mud snails and planarians. The apparatus, which is essentially the same for both animals, consists of a small shallow, water container centered over a polar coordinate grid. The orientation of the organism is recorded as its position when it passes over the grid. The apparatus is placed in a box which furnishes a constant light field, and can be rotated to face any compass direction. Bar magnets may be placed in slots beneath the polar grid to augment, reverse, or otherwise modify the horizontal component of the natural magnetic field. Experiments of this type were begun in 1959 by a group of investigators led by Frank A. Brown, Jr. Their reports, summarized in the following paragraphs, indicate that the nature of the response of these organisms varies as a function of such factors as (1) geographic orientation of the organism in the earth's own magnetic field, (2) strength and direction of experimental horizontal magnetic vectors, and (3) natural solar and lunar cycles.

Orientation in the Geomagnetic Field

According to Brown and Webb (1960), the marine mud snail, *Nassarius obsoletus*, appears to distinguish among the four compass directions while being tested in the earth's magnetic field. The data reveal a mean path characteristic for each direction (north, south, east, and west) as the snails emerge from a uniformly illuminated corridor at the same time everyday. This compass-directional phenomenon seems to possess a monthly modulation. Brown and Barnwell (1961) assayed paths of snails initially directed in eight angular relationships to the horizontal component of geomagnetism from 0° to 270°. Their findings indicate a progressively greater left-turning when the magnetic axis was at 45°, 135°, 225°, and 315°, rather than at adjacent parallel or right angle positions. Other studies, conducted in both symmetrically (Brown, Brett, Bennett, and Barnwell, 1960) and asymmetrically (Barnwell and Brown, 1964) illuminated fields, indicate that orientation of snails does indeed include a true response to the earth's magnetic field. Experiments with <u>Dugesia</u> (Brown, 1962a, 1962b; Barnwell and Brown, 1964) show that a compass-direction effect is present in planarians also. The worms exposed only to the earth's field clearly distinguished between north-south and east-west orientations of the apparatus, with right-turning when directed either north- or southward and left-turning when directed either east- or westward.

<u>Effects of Weak Experimental Magnetic Fields</u>

The effects of both strength and direction of weak experimental fields, produced by placing magnets beneath the orientation chamber, have also been studied.

It has been demonstrated that <u>Nassarius</u> is able to perceive small changes in strength of this horizontal component ranging from about 2 gauss (ten times that of the earth) to 10 gauss (Brown, Brett, Bennett, and Barnwell, 1960; Brown, Webb, and Brett, 1960; Barnwell and Webb, 1961). The mean amount of turning, whether clockwise or counterclockwise, shows a significant increase in these experimental fields over that of the earth, and also displays daily and monthly rhythms. Exposing snails to experimentally reversed fields, Brown and Barnwell (1960) report that right-turning is induced at the time of full moon when the strength of the experimental field differs from the strength of the earth's field by no more than a factor of 4. Right-turning is induced at the time of new moon when the strength of the reversed field is greater than 4 times that of the earth. Planarians also have been reported to distinguish differences in strength of experimental horizontal magnetic fields (Brown, 1962, 1962b; Barnwell and Brown, 1964; Brown and Park, 1965). The findings reveal that in going from strengths of 0.25 to 5.0 gauss, a north-directed field clearly induces increased left-turning. Between strengths of 5.0 and 10.0 gauss, the direction of induced turning is reversed.

If orientation in the magnetic field is to be useful in navigation, organisms should be able to distinguish directions of the lines of magnetic force, in addition to strength differences. This ability has been observed in mud snails as differential responses to experimental fields at right angles to one another (Brown, Webb, Bennett, and

Barnwell, 1959; Brown, Bennett, and Brett, 1959; Brown, 1960; Brown, Bennett, and Webb, 1960; Brown and Barnwell, 1961; Barnwell and Brown, 1964; Brown, Webb, and Barnwell, 1964). The snail distinguishes parallel from right-angle horizontal orientations of these experimental fields relative to its body axis. Rotation of a 1.5-gauss horizontal field produces orientational behavior correlated with that observed when the snails are rotated in the opposite direction in the earth's field. Rotation of a 5-gauss field may produce a pattern either paralleling or mirror-imaging that of the earth. The differences between the parallel and right-angle fields systematically vary according to solar-daily, lunar-daily, and monthly rhythms. Dugesia clearly differentiate between parallel and perpendicular fields in a manner similar to, but more pronounced than, that in snails. The relationship between direction of experimental field and worm-turning becomes steadily stronger in passing from the earth's field to a 5-gauss field, but between 5-gauss and 10-gauss fields there is an abrupt reversal of the sign (Brown, 1962a, 1962b; Barnwell and Brown, 1964). The fact that this response alters its character in passing from the earth's field to one as small as 10 gauss, suggests that the perceptive mechanism may be specifically adapted to such a weak field as the geomagnetic one. Other experiments (Brown, 1962a, 1962b), in which a 5-gauss field is rotated at each of the seven 15°-intervals from north to west, indicate that the worms can resolve horizontal field direction with remarkable precision. The planarian response patterns also exhibit diurnal, monthly, and annual rhythms.

Effects of Solar and Lunar Cycles

As mentioned several times above, extrinsic rhythms play an important part in affecting the orientational responses of snails and planarians. The solar-day (24 hours), the lunar-day (24.8 hours), and their derivative, the 29.5-day synodic month, all seem to be responsible for observed fluctuations in the responses of these organisms.

Daily rhythms have been observed for both snails and planarians (Brown, 1960; Brown, Webb, and Brett, 1960; Brown, Bennett, and Webb, 1960; Brown, 1962a; Barnwell and Brown, 1964) tested in the earth's field and in the presence of weak artificially-induced magnetic fields. The solar-daily and lunar-daily variations are strikingly similar both in gross features and phase relationships. At both sunrise and moonrise, left-turning is minimal but generally increases again as these bodies set. Also, the standard deviation of pathways is minimal about the time of sun- and moonrise and sun- and moonset, and gradually reaches a maximum while sun and moon are above the horizon.

Experimental magnetic fields, augmenting that of the earth, seem to strengthen this response. Since the mean path of the organisms is related to the positions of the sun and moon, the increased magnetic flux causes them to orient themselves more effectively. Brown (1960) suggests that organisms displaying these daily rhythms possess a receiving system which has two sets of "directional antennae"—one geared to the solar day and one to the lunar day.

These two similar daily rhythms would be expected to produce, by periodic interference, longer-term variations whose periods reflect the 29.5-day synodic month. Such monthly cycles have been demonstrated in both snails and planarians. In each of two consecutive synodic months, snails exhibit maximum right-turning when north-directed and minimum right-turning when south-directed during the fortnight centered on full moon. For the alternate fortnights, those centered on new moon, the pattern was bimodal and of approximately half the amplitude, with maxima in right-turning when either north- or south-directed and minima when east- or west-directed (Brown and Webb, 1960). Snails exposed to an experimental field approximately ten times that of the earth also display a conspicuous rhythm. Experimentals turn to the right of controls on days just prior to new moon and full moon and maximally left of controls near the times of the moon's quarters (Brown, 1960;

Brown, Webb, and Brett, 1960; Barnwell and Brown, 1964). Planarians, directed initially northward in the late morning hours in an unvarying pattern of illumination, exhibit a synodic monthly rhythm which appears to undergo an annual modulation (Brown, 1962a). From late August to March, the worms veer maximally to the left at new moon and to the right at full moon. During March and April a semi-monthly pattern gradually develops with right-turning at both new and full moon and left-turning at the moon's quarters. This pattern then tends to reverse itself and mean paths remain relatively scattered during the summer months. Thereafter, there is a gradual return to the clear monthly fluctuation with maximum left-turning at new moon and right-turning at full moon.

Response to an experimental field about twenty-five times the geomagnetic field strength manifests itself in substantial alterations of synodic monthly cycles in planarians. An east-west oriented field reduces the amplitude of the cycle and a north-south oriented field abolishes the cycle (Brown, 1962a; Barnwell and Brown, 1964). Another study (Brown and Park, 1965) reveals that it is possible to shift the phase of a lunar rhythm in planarians by experimentally reversing the horizontal magnetic vector. In conditions where control worms displayed maximum left-turning just prior to new moon, these experimentals exhibited a shift in phase with maximum right-turning just prior to new moon. Brown and Barnwell (1960) have also reported the effect of reversed fields on the monthly rhythms in snails. Even though much evidence has been advanced supporting the hypothesis that orientation is influenced by biological clocks, these studies seem to give the first evidence that a biological rhythm itself can have its phase reset by altering the vector angle of a geographical field component.

Other Factors Affecting Geographic Orientation

There seems to be little reason to doubt that spatial orientation of snails and planarians, expressed as an amount of turning, is affected by weak magnetic fields and possesses a definite rhythmicity. The problem of analyzing these responses to magnetism is compounded by recent discoveries indicating that these responses can be influenced by other closely-related geophysical factors. Mud snails (Webb, Brown, and Schroeder, 1961) and planarians (Brown, 1962c) seem to be extremely sensitive to differences in electrostatic fields. Brown (1960) also reports a striking similarity between a lunar-day cycle of magnetic response in snails and a simultaneous spontaneous activity cycle of mice. Definite correlations between oxidative metabolic changes in snails and their magnetic responses have also been reported (Barnwell, 1960). Magnetic orientations, cellular oxidations, and spontaneous activity cycles all show similarities to barometric pressure changes. Because organisms do respond to such subtle geophysical factors, biologists must investigate changes in other, hitherto ignored, factors which may also be reflected in fluctuations within living systems. We can see, clearly, that the perceptive mechanism for weak magnetic fields is not isolated from the remainder of the living organism, and that we are dealing with a widely distributed biological phenomenon.

III. Effect of Magnetism on Learning in Planarians

A review of the literature reveals no references to studies involving the effects of magnetism on learning in planarians. Best (1964) has reported two instances of a relationship between learning and lunar cycles which could possibly be related to such a magnetic effect.

First, Best cites the results of a study, conducted in 1962, in which planarians were trained to criterion in a T-maze. A criterion session was one with no errors. He found that subjects tended to produce criterion sessions within a few days of one another regardless of the amount of training they had had. These criterion session times tended to recur within a period approximating a lunar month, and to slightly lag the time of the full moon.

Secondly, Best (1964) reports a semi-lunar cycle in, what he terms, "reminiscence behavior". The instrumental conditioning apparatus previously described (where planarians are trained to intercept a beam of light with a period of darkness as the reward) was used here. The worms were given two seven-hour training periods separated by a 39-hour rest period in darkness. Best found that some time between the close of the first session, in which there was no evidence of learning, and the beginning of the second (a period in which there was no opportunity for contact with the training situation) the worms learned, insofar as the difference in rate of responding between experimental and control can be considered to be a measure of learning. This apparent learning during a period of no overt practice is what Best calls "reminiscence effect". When the difference between experimental and control animals is plotted against the time of lunar month, a definite semi-lunar cycle is evident. Experimental subjects show a significantly greater number of correct responses than control subjects during the times of new and full moon. During the period centered over half moon, experimental subjects show significantly fewer correct responses than control subjects.

The rhythms reported here are not to be interpreted as resulting directly from a magnetic effect but are mentioned only as examples of observed periodicities in learning. Fluctuations in the geomagnetic field, as well as many other geophysical factors in the environment, seem to be directly related to lunar periods.

METHODS AND MATERIALS

Experiment I - Instrumental Conditioning

Subjects. The subjects were 60 fresh-water planarians chosen at random from four colonies of approximately 50 worms each. These were identified as *Dugesia tigrina* by Powell Laboratories, Gladstone, Oregon, and were received on December 10, 1966. The colonies were housed in darkness in glass finger bowls filled with aerated, filtered, creek water at temperatures of 70° to 75° F. Worms received fresh water daily and were fed raw beef liver twice weekly. During its seven-day training period, each subject was housed individually, also in darkness, in a small glass jar with water at a depth of approximately two inches. Subjects received fresh water following training each day and were not fed during this period.

Apparatus. The apparatus consisted of a Y-maze placed in a small, black, wooden box (8" x 8" x 4", open at the top). The maze was continuously illuminated through a 0.5-inch circular opening cut in the rear of the box, approximately one inch from the bottom. A 7½-watt opalescent bulb, mounted behind this opening on the outside of the box, provided the illumination (Figure 1A). This bulb was covered by a black curtain from above, so light would reach the maze only through the small opening. A small, stationary platform was constructed in the floor of the box

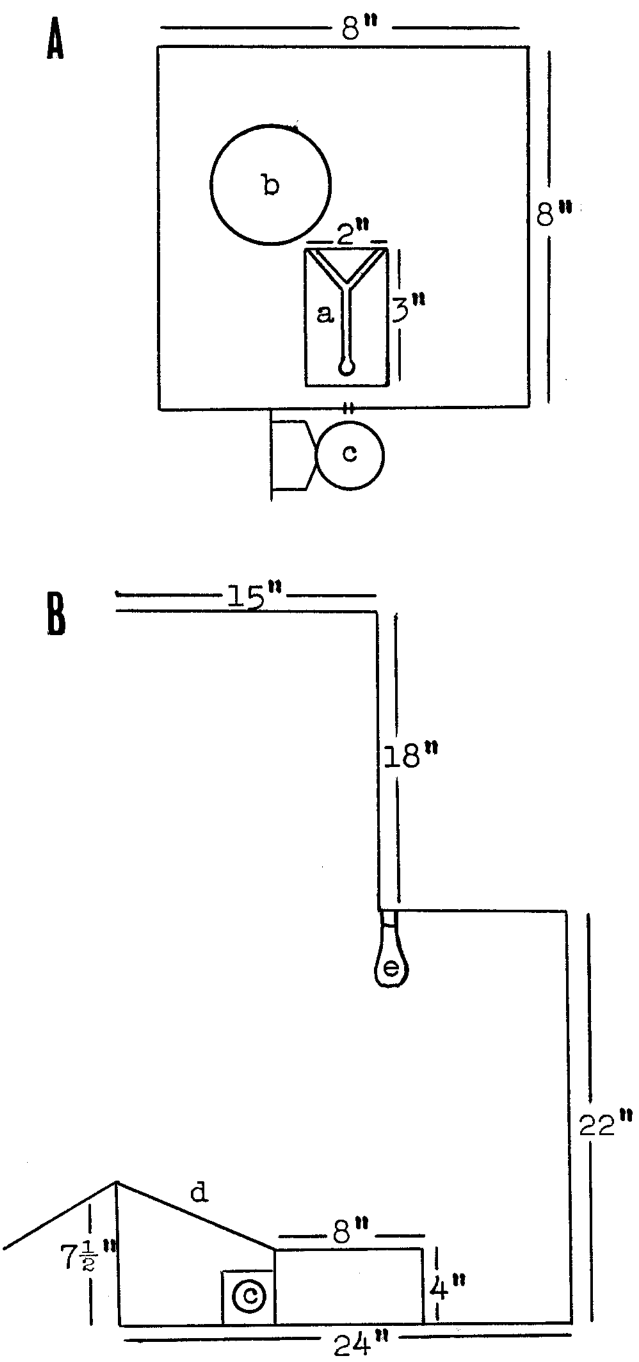


Figure 1. Instrumental conditioning apparatus. A-Top view of small box containing Y-maze. B-Lateral view of diagrammatically-sectioned large wooden box drawn to scale (1" = 10") with maze apparatus in position. (a- Y-maze; b-dish for subjects receiving noxious stimulus; c- 7½-watt bulb; d-curtain to shield maze from weak light source; e- 100-watt bulb)

to raise the maze to within one-half inch of the posterior illumination.

The Y-maze was formed from three, 1/8-inch deep, V-shaped grooves cut in a piece of transparent plexiglass (2" x 3" x 3/16"). A circular well, continuous with the stem of the Y, was cut to act as a reservoir for excess water. During testing, the maze was centered in front of the rear light source on the platform.

Even though the laboratory itself was in semi-darkness, the entire Y-maze apparatus was placed in a large, black-lined, wooden box (24" x 15" x 22") to eliminate any excess light during experimentation. The top of this larger box was equipped with a 100-watt bulb mounted on the inside, 16 inches above the level of the maze (Figure 1B). This bright light source, which acted as the noxious stimulus during instrumental conditioning, was fitted with a switch so it could be operated rapidly. Through a shielded, curtained opening above and behind the maze, animals were observed and manipulated. In operation this large box remained stationary on a table 28 inches above the floor.

All studies were conducted in Vancouver, Washington (45° 38' N; 122° 41' W; altitude, 26 feet). The laboratory itself was in darkness except for a lamp with a 50-watt bulb placed on the table beside the larger box. This provided only enough stray indirect light inside the box to allow the subject to be seen by the observer, and it remained unchanged throughout the period of experimentation.

Procedure. Maze learning is an example of instrumental learning, where the animal is punished for choosing the wrong arm of the maze or rewarded for making the correct choice. In order to eliminate the possible chances of overpunishment and physiological damage caused by shock, a period of exposure to bright light was chosen as the punishment in this study. Punishment was administered immediately following the incorrect choice, rather than being present at all times except during the reinforcement period, as in the experiments of Best and Rubinstein (1962) and Lee (1963). The reward here consisted of the prompt return of the subject to its home bowl for a rest period in the darkness.

To investigate possible effects of the geomagnetic field on learning, the apparatus was rotated by 90° intervals in the earth's field. Subjects were treated in four groups, each with the apparatus facing a different compass direction (East, South, West, or North). Each group of 15 worms received seven consecutive days of training during the course of the four-week study. For the first week (Jan. 10-Jan. 16; new moon to first quarter) the apparatus faced East; the second week (Jan. 17-Jan. 23; first quarter to full moon), South; the third week (Jan. 24-Jan. 30; centered on full moon), West; and the fourth week (Jan. 31-Feb. 6; last quarter to new moon), North. In each group, five worms were reinforced to choose the right arm of the maze; five were reinforced to choose the left arm; and five served as controls. The controls received reinforcement following every trial. For the purposes of recording and identification, the 15 worms in each group were assigned letter codes, according to the reinforcement they received (R_1, L_1, C_1; R_2, L_2, C_2; R_3, L_3, C_3; R_4, L_4, C_4; R_5, L_5, C_5).

Each subject underwent seven trial sets, one on each day of its training period. A trial set consisted of 15 trials in the maze, followed by the subject's return to its home bowl and darkness until the following day.

Prior to the testing period each day, naive planarians were allowed to crawl randomly in the Y-maze, covering all surfaces with residual mucous trails. This was done to prevent possible response patterns resulting from the use of past trails as cues.

Each daily testing period lasted from about 9:30 until 5:00. The subjects received training in five small groups of three worms each. Group 1 (R_1, L_1, and C_1) was always tested first in the day, followed by Group 2 (R_2, L_2, and C_2), etc. Approximately 90 minutes were required to administer 15 trials each to the three subjects in a group. The three worms were introduced into the maze in a definite sequence (R_1-trial 1, followed by L_1-trial 1, C_1-trial 1, R_1-trial 2, L_1-trial 2, C_1-trial 2, R_1-trial 3, etc. for 15 trials). This same sequence was repeated for groups 2, 3, 4, and 5 until each of the 15 worms had received 15 trials.

One complete trial will be discussed to illustrate the training procedure which was followed. R_1 is introduced into the water-filled maze, approximately one inch behind the choice point. Because this subject is reinforced to select the right arm of the maze, choice of that arm would be followed by immediate return to the home bowl for a five-minute rest period under a black curtain outside the large box. The choice of the left alternative by R_1 would be followed by immediate removal from the maze to a small, white, plastic dish for a 90-second exposure to the bright overhead light. Following this 90-second period, the subject is moved (still in the white dish) outside the box for a 3½-minute wait in the light provided by the lamp on the table. During the beginning of the rest period of R_1 (whether in darkness or light), L_1 is introduced into the maze. This subject is reinforced to choose the left arm of the maze, with correct and incorrect choices treated exactly the same as for R_1. During the beginning of the rest period of L_1 (whether in darkness or light), C_1 is introduced into the maze. This subject is reinforced regardless of the arm of the maze it chooses. Following choice it is transferred to its home bowl outside the apparatus for a five-minute rest period in the dark. During the beginning of the rest period of C_1, R_1 is again introduced into the maze; this time for its second trial. The same sequence is repeated for all succeeding trials.

The responses observed for the total of 225 trials administered to the 15 subjects each day were recorded on a single data sheet (Appendix 1). For the experimental subjects (those reinforced to select the right or left arm of the maze), a correct response was recorded as a (+) and an incorrect response as a (-). The responses of control subjects were recorded as right (R) or left (L) turns. A (0) was recorded if the subject refused to "run" the maze. This included responses such as crawling away from the choice point, hesitation at the choice point followed by a complete reversal in direction, and a complete refusal to move in the maze. Subjects were returned to home bowls placed in the light following responses of this type.

Throughout the procedure, all subjects received equal amounts of handling, which was kept at as low a level as possible. An eye dropper with a large opening at the tip (2.5mm.) was used for all transferring operations to avoid injuring the worms. Other than the transfers between

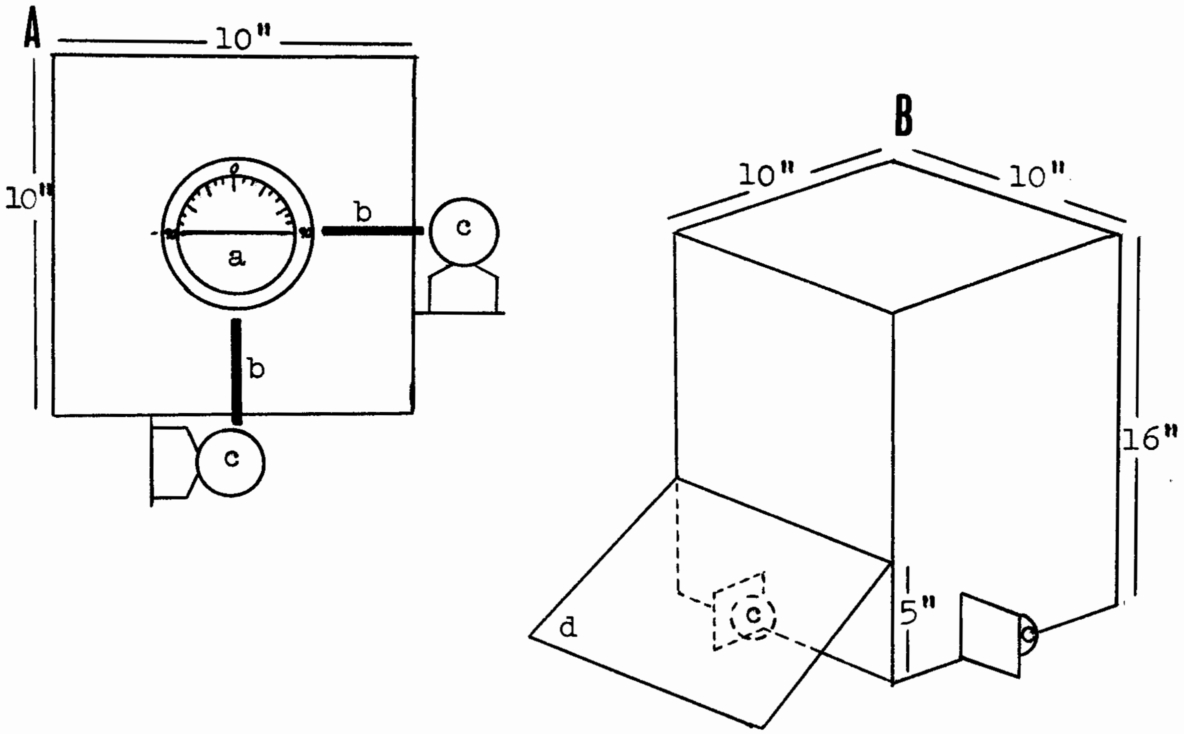
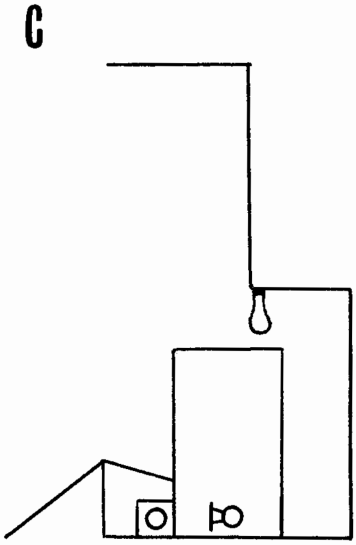


Figure 2. Orientation apparatus. Top (A) and lateral (B) views of cabinet containing orientation apparatus. (a-Petri dish centered over polar coordinate grid; b-sleeved light-conducting glass tubes; c- 7½-watt bulbs; d-curtain to shield dish from light)



Lateral view (C) of diagrammatically-sectioned large wooden box with orientation cabinet in position.

home bowl and maze during training, no further handling of subjects was required.

Experiment II - Spontaneous Orientation Reactions

Subjects. The subjects were 28 Dugesia tigrina chosen from the same colonies as those in Experiment I. During its seven-day observation period, each subject was housed individually in a small glass container. The worms were kept in darkness, received fresh water daily, and were not fed during this period.

Apparatus. The orientation apparatus, similar to that used by Brown (1962a), consisted of a 3 3/4-inch glass Petri dish centered over a polar coordinate, paper grid (Figure 2A). This apparatus was set inside a black-lined wooden cabinet (10" x 10" x 16"), open at the top of the back for manipulation and observation (Figures 2A and 2B). The apparatus was continuously illuminated by two weak horizontal sources; one parallel to the zero axis of the grid and the other parallel with the 90° axis from the right side. This illumination pattern was adopted because Brown (1962a) found the variance of paths to be less in an asymmetrical field of this type. The horizontal light sources were black-sleeved, 10-mm. solid glass rods, covered on the ends with onion-skin paper, conducting light into the cabinet from two 7½-watt opalescent bulbs attached to the outside of the cabinet. A black curtain was fastened beneath the opening in the rear of the cabinet so light could reach the dish only through the glass tubes.

To minimize any stray light from the laboratory, this cabinet was placed inside the same large, black, wooden box used for Experiment I (Figure 2C). During the experiment, the box remained stationary on a table 28 inches above the floor.

The use of ferromagnetic materials was carefully avoided in the construction of the entire apparatus.

Procedure. The 28 subjects were divided into four groups, and the reactions of each group were observed with the apparatus facing a different compass direction in the earth's magnetic field. This study was conducted concurrently with Experiment I. The E-group (seven worms tested with apparatus facing compass East) was tested the first week (Jan. 10-Jan. 16); the S-group the second week (Jan. 17-Jan. 23); the W-group the third week (Jan. 24-Jan. 30); and the N-group the final week (Jan. 31-Feb. 6). Each day 14 planarian paths were recorded, a morning and an evening response for each of the seven subjects. The morning testing period was always between 9:00 and 9:30 and the evening period was always between 5:00 and 5:30 to minimize the effect of any daily variation.

In operation the planarian is transferred from home bowl to center of the Petri dish and quickly oriented with the tip of the eye dropper toward the zero axis of the polar grid. The deviation in worm path from the initial direction is then recorded in terms of the point, to the nearest 5°, at which the worm crosses the circular arc one inch from the origin. An (X) was recorded in the few instances in which the subject crawled away from the arc or refused to move at all. The paths observed for each week of testing were recorded on a single data sheet (Appendix 2).

Due to the number of observations included in this study, it was felt that it would be appropriate to make a computer analysis of all data involved. The results of both experiments were programmed at the Computer Center, Central Washington State College.

RESULTS

Experiment I - Instrumental Conditioning

In previous instrumental learning paradigms (Best and Rubinstein, 1962), researchers have described "learning" in terms of a curve relating the proportion of correct responses exhibited by experimental subjects in each trial set. The results of the present study are shown in Figure 3 indicating mean performance in terms of the number of correct responses divided by the total number of responses in that trial set. Statistical analyses describing these learning curves follow. The figures used in calculating these quantitative measures of difference are listed in Table 1.

An increase in the mean proportion of correct choices can be seen in Trial Sets 2 and 3 over that in Trial Set 1 (Figure 3). To test whether there is actually an initial phase in which learning occurs, the mean performance on Trial Sets 2 and 3 can each be compared with that in Trial Set 1. The results of these t tests are given in Table 2. It can be seen that none of these t values is large enough to suggest an initial learning phase, even though the learning curves in Figure 3 seem to indicate this.

Applying the sign test, a less precise one, to these same data offers some slightly different results in

Trial Set	1		2		3		4		5		6		7	
	x	S.D.	x	S.D.	x	S.D.	x	S.D.	x	S.D.	x	S.D.	x	S.D.
East-oriented	.468	.167	.571	.174	.682	.099	.531	.197	.481	.138	.719	.142	.574	.187
South-oriented	.402	.139	.513	.202	.521	.247	.504	.251	.465	.201	.581	.232	.643	.297
West-oriented	.414	.157	.461	.154	.595	.196	.510	.248	.577	.181	.530	.215	.490	.207
North-oriented	.489	.168	.589	.101	.616	.201	.569	.200	.565	.164	.524	.236	.416	.215
Combined	.443	.156	.534	.165	.604	.195	.529	.218	.522	.172	.588	.217	.531	.238

Table 1. Mean proportion and standard deviation of correct responses in each trial set.

	T.S. 1 vs. T.S. 2	T.S. 1 vs. T.S. 3
E-oriented	.40	.76
S-oriented	.48	.47
W-oriented	.21	.72
N-oriented	.38	.45
Combined	.25	.42

Table 2. *t* values comparing mean proportion correct responses of trial sets indicated.

	N	Trial Set 2	Trial Set 3	Trial Set 2 or 3	Trial Sets 2 & 3	At least 2 t. sets	At least 3 t. sets	At least 4 t. sets	At least 5 t. sets	At least 6 t. sets
East	10	7	9*	9*	7	10*	9*	6	5	2
South	10	6	7	9*	4	8	8	8	6	3
West	9	6	9*	8	5	9*	7	7	5	1
North	10	7	7	8	6	7	7	6	5	3
All	39	26*	32*	35*	23	34*	31*	27*	21	9

Table 3. Number of subjects of the N experimentals which had better performances on trial sets indicated than on Trial Set 1. Starred (*) entries are significant values ($p < .05$) on a one-tailed sign test.

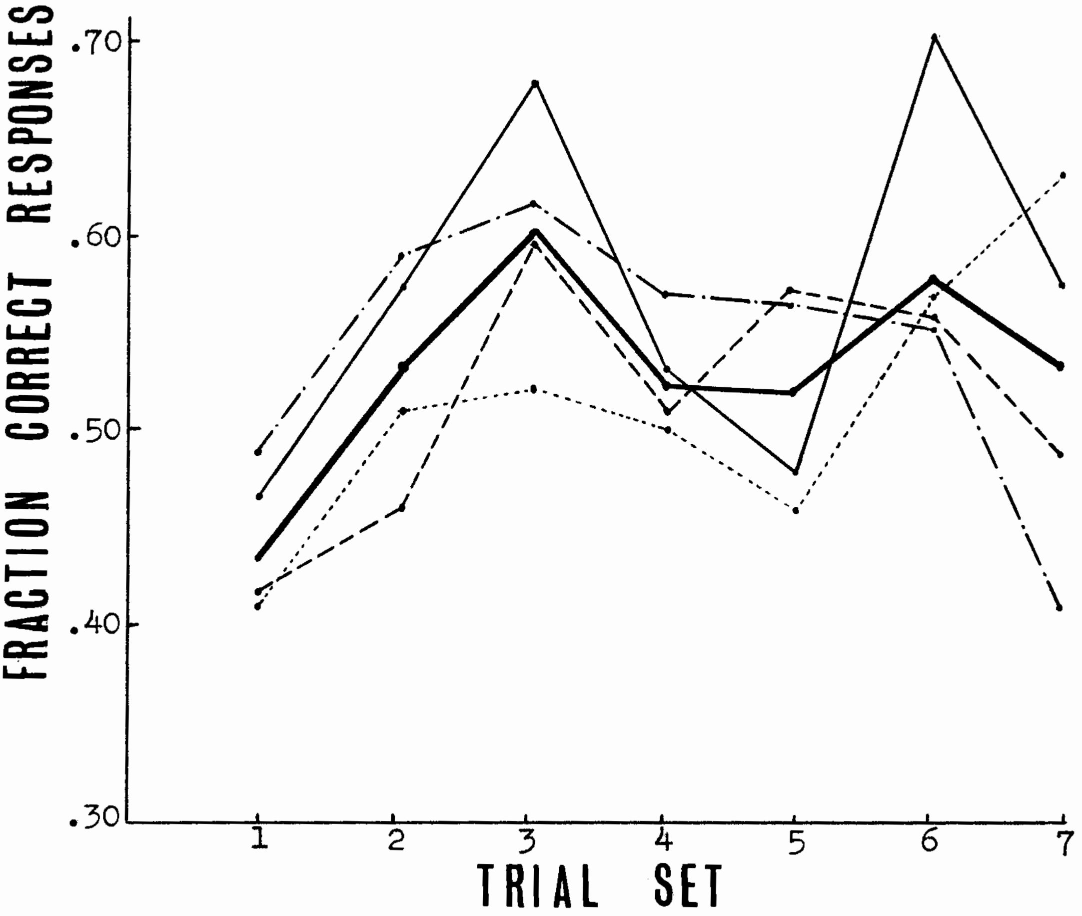


Figure 3. Mean proportion of correct responses in each trial set, for each orientation of the apparatus and for all orientations combined.
(East ——, South ·········, West - - - -, North —·—, All ━━)

describing the learning curves. The figures used in the sign tests are listed in Table 3, indicating the number of the N experimental subjects in each group which had better performances on the trial sets listed than on Trial Set 1. The starred (*) entries are those values which are significant ($p<.05$) on a one-tailed sign test. There is no difference between right- and left-reinforced groups in these figures. In other words, each value listed in Table 3 includes approximately equal numbers of right- and left-reinforced experimental subjects.

Considering all orientations combined, Table 3 shows that of the 39 experimental subjects completing seven trial sets, a significant ($p<.05$) number gave higher proportions of correct responses on Trial Set 2, Trial Set 3, Trial Set 2 or 3, and at least four trial sets than on Trial Set 1. The following three comparisons will allow a closer examination of the initial phase of the learning curve. Sign tests are used to compare performances on Trial Sets 2 and 3 with Trial Set 1, and also Trial Set 2 with Trial Set 3. It is assumed that the chances of obtaining either a higher or a lower proportion of correct responses on a given trial set compared to the previous trial set are equal. First, of the 38 subjects which had either a higher or lower proportion of correct responses on Trial Set 2 than on Trial Set 1, 26 had better performances, and 12 had a lower proportion correct. When compared to a ratio of 0.5, this gives $p<.01$, considered to be highly significant on a one-tailed sign test. Secondly, comparison of the Trial Set 3 performances with Trial Set 1 indicates an even greater increase in choice of the reinforced alternative. Of the 39 subjects which had either a higher or lower proportion of correct responses on Trial Set 3 than on Trial Set 1, 32 had better performances and 7 had a lower proportion correct. This gives $p<.0001$, highly significant on a one-tailed sign test. Thirdly, 24 subjects had better performances on Trial Set 3 than on Trial Set 2, and 14 had a lower proportion correct. When compared to an expected proportion of 0.5, this proportion gives $p<.05$, regarded as significant on a one-tailed sign test.

The sign test applied to each of the four separate orientation groups also results in some instances of performances significantly better than naive (Trial Set 1) scores. In the E- and W-oriented groups, a significant ($p<.05$) number of subjects exhibited better performances on Trial Set 3 and on at least two trial sets than on Trial Set 1. The S- and N-oriented groups failed to show significantly higher ($p>.15$) proportions in these categories.

It can be noted from Figure 3 that the trial set at which the mean peak performance occurs varies with each orientation group. An examination of individual learning

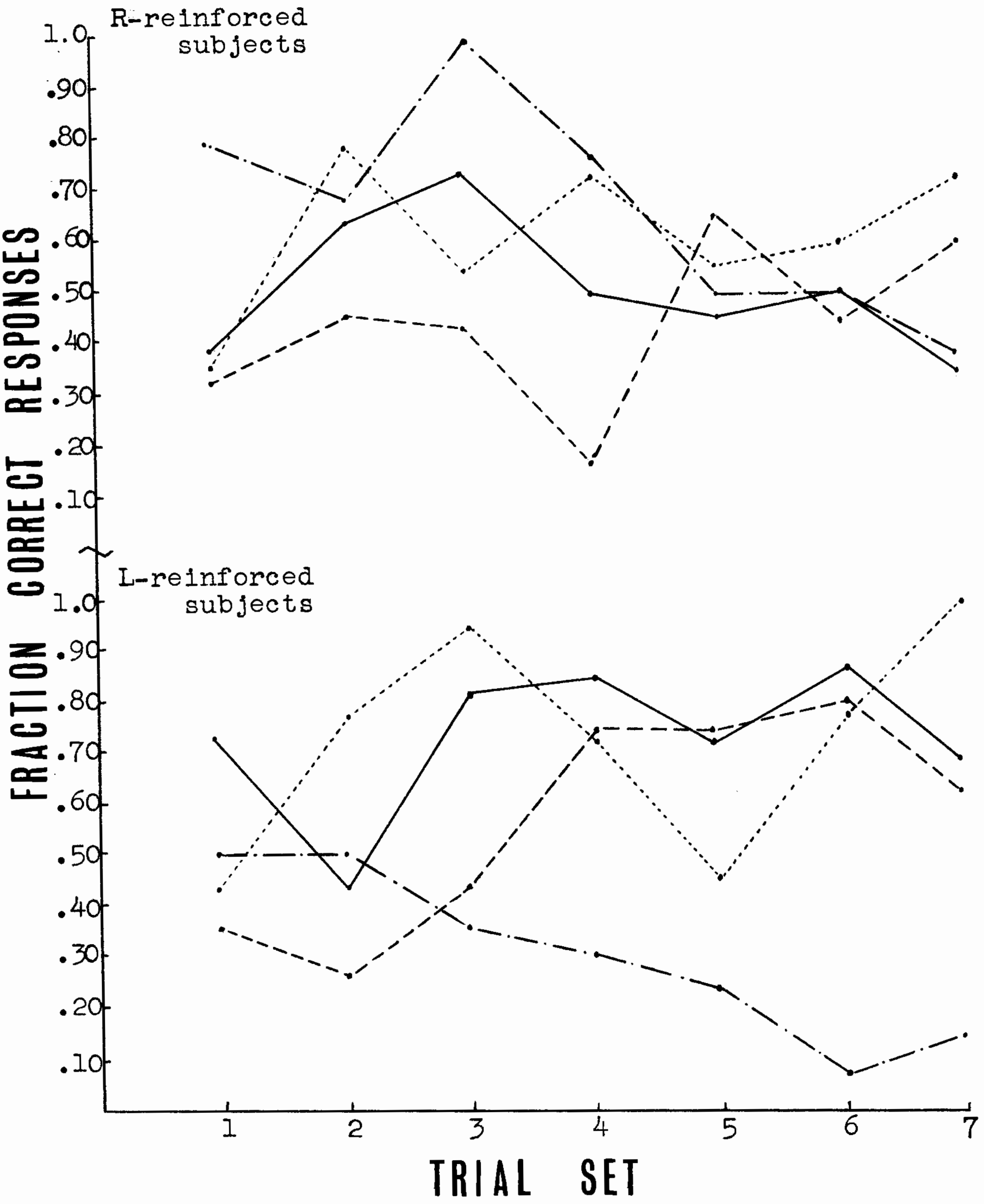


Figure 4. Individual learning curves for 8 subjects (R_1 and L_1 for each orientation) expressed as proportion of correct responses in each trial set.
(East ———, South ········, West – – – –, North —·—·)

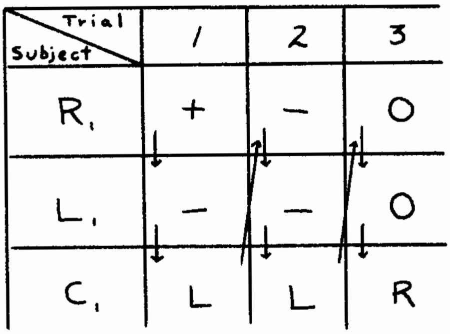
curves and also the large standard deviations (Table 1) reveals that the trial set at which the peak performance occurs also varies greatly among individual subjects. This is illustrated by Figure 4 which shows the individual performances of eight subjects (subjects R_1 and L_1 for each orientation). In a situation such as this, with large variances in individual performance, group averages tend to become meaningless. The mean of the peak performances of the 38 subjects exhibiting at least one trial set with a better performance than Trial Set 1 is 0.79.

Best and Rubinstein (1962) reported one characteristic of the learning curve which was not duplicated at all in this study. They reported a significant ($\underline{t}= 2.55$, $p<.05$) drop in maze performance in the trial set immediately following the attainment of the peak. The reported active rejection of the reinforced alternative actually overshot to a value lower than either the naive score or that which could be accounted for by chance. The subjects included in a test of this type must have some trial set with a performance higher than that on the first, and have a trial set following that set on which the maximum performance was attained. In the present study, 32 subjects met this criterion. The mean (proportion correct responses) of the first trial set for these subjects is 0.454 and the mean for the trial set immediately following the high performance set is 0.552. Therefore, the abrupt drop in performance to a level lower than the naive level was not present in this study, although a striking drop was noted.

Several tests were applied to determine whether subjects exhibited any preference for one arm of the Y-maze over the other. First, comparing the mean number of correct responses on Trial Set 1 (naive performance) of the right-reinforced subjects with that of the left-reinforced subjects gives a $\underline{t}$ value of .1193, showing no significant difference. Secondly, a $\underline{t}$ test was used to compare the mean difference in increase of correct response of the right- vs. the left-reinforced subjects. The increase in correct response for each subject was designated as the number of correct responses in Trial Sets 2 plus 3 minus the number of correct responses in Trial Set 1. The results, $\underline{t}=.0040$, indicate no significant difference in increase of correct response between the right- and left-reinforced groups. A third test compared the proportion of choices for each arm of the maze for all the control subjects. Of the 2081 total right or left responses of the controls, 1017 were toward the right arm and 1064 were toward the left arm. A chi square test, $\underline{x}^2= 1.06$, $p>0.3$, shows no significant difference between this proportion and 0.5, which would be expected by chance.

Because the experimenter observed that subjects often tended to follow or repeat previous responses, an analysis of these data was also included. This was to determine whether the worms were following the paths of subjects introduced into the maze just previously, following their own path from the previous trial, or if choice was completely independent of previous responses.

To accomplish this, each response was given a code number indicating how this particular response compared to the three previous ones. The number of observations in each code group could then easily be counted. Because the worms were introduced into the maze in groups of three (R_1, L_1, C_1, etc.), descriptions of the three previous responses would be sufficient to show whether a subject was following its own path or that of either of the other two subjects. The following portion of a data sheet and the method of coding the responses will serve as an example:



Trial / Subject	1	2	3
R_1	+	−	O
L_1	−	−	O
C_1	L	L	R

<u>trial 2</u>:

R_1-follows previous subject

L_1-follows self

C_1-follows self and penultimate subject

<u>Sample Data Sheet</u>
(arrows indicate order in which subjects were introduced into maze)

> In trial 2, worm R_1 chose the left arm of the maze, as did only the immediately preceding worm (C_1). Worm L_1 (trial 2) chose the right arm of the maze, as it itself did on trial 1. Worm C_1 (trial 2) chose the left arm of the maze, as did the penultimate worm (R_1-trial 2) and itself (C_1-trial 1). The "penultimate worm" is a term used to describe the subject introduced into the maze two ahead (prior to the immediately preceding worm) of the subject in question.

The assigned codes allowed for the fact that in some cases a response was not preceded by three responses, as occurred if one or both of the two preceding subjects failed to make a choice (0) on that trial. This was done to make the analysis more accurate. For example, if we assigned a code merely indicating following response to the trial 3 response of worm C_1 (see above), it would denote that this worm followed none of the previous responses. It would seem that C_1 chose the opposite arm of the maze from R_1 and L_1 in trial 3, when actually these two responses were not even present. Therefore, in this case, a code was assigned showing that (a) no following occurred, and (b) only the response of this worm itself was present. The various codes, which take into account all the possible following responses and also the previous responses which were absent, will not be enumerated here.

Table 4 gives the values used in tests to determine the extent of following previous paths. Chi square tests were administered to compare the proportions obtained with

Type of Following	# Responses
Follows immediately previous response	3483
Does not " " "	3171
Follows self on previous trial	3278
Does not " " "	2746
Follows penultimate response	2736
Does not " " "	2800
Follows 3 previous identical responses	883
Does not " " "	698

Table 4. Frequencies of various following responses.

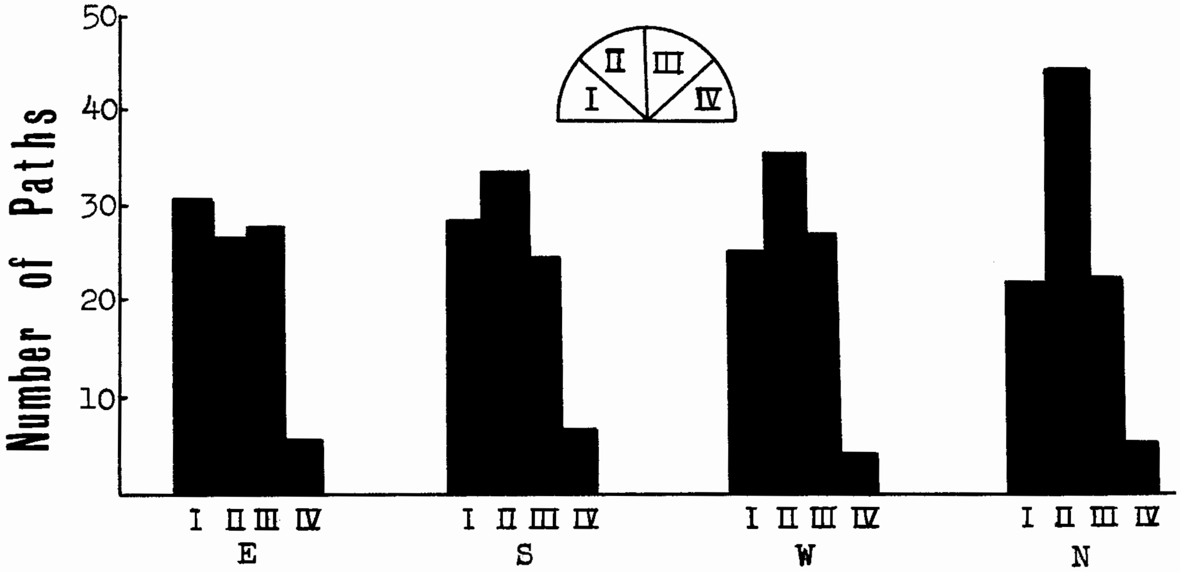


Figure 5. Frequency distribution of planarian paths in each quadrant of the polar coordinate grid for each compass direction.

those which would be expected by chance.

There seems to be a slight tendency for planarians to follow the path of the immediately preceding subject in the maze. Of 6654 maze choices where this preceding response was present, 3483 followed and 3171 chose the alternative arm of the maze. When compared to an expected proportion of 0.5, this gives $\underline{x}^2 = 14.63$, $p < .001$.

The path of the worm itself on the previous trial also seems to influence the next response. Of 6024 responses where the subject in question had a response on the trial ahead, 3278 repeated this response and 2746 did not. When compared to an expected proportion of 0.5, this gives $\underline{x}^2 = 46.98$, $p < .0001$.

The response of a previous subject seems to have no effect on the response of a following subject when another worm is introduced into the maze between these two. Of 5536 responses where the penultimate (two ahead) response was present, 2736 followed this and 2800 did not. When compared to an expected proportion of 0.5, this gives $\underline{x}^2 = 0.74$, $p > 0.35$ (not significant).

A comparison of the frequencies of paths which repeat the self-response with those repeating the immediately preceding one reveals a slightly greater tendency for following the self-response. A sample size of 3000 (1632 follow self, 1368 do not; 1569 follow previous, 1431 do

	N	x	S.D.	S.E.
E-oriented				
AM	46	-24.6°	42.6	6.3
PM	46	-12.2°	49.4	7.3
Combined daily	92	-18.4°	46.3	4.8
S-oriented				
AM	47	-11.3°	35.9	5.2
PM	49	-16.2°	45.0	6.4
Combined daily	96	-13.8°	40.7	4.2
W-oriented				
AM	49	-15.2°	40.6	5.8
PM	48	-18.3°	38.9	5.6
Combined daily	97	-17.0°	39.6	4.0
N-oriented				
AM	49	-15.3°	42.2	6.0
PM	44	- 9.6°	34.3	5.2
Combined daily	93	-12.6°	38.6	4.0

Table 5. Mean planarian paths and deviations for morning, evening, and combined daily observation periods.

	A	B	C	
	Total # Correct Responses of R-reinforced Ss	Total # Correct Responses of L-reinforced Ss	Total # R and L Responses of Control Ss	
			R	L
N- and S-oriented	505	516	516	525
E- and W-oriented	510	516	501	539

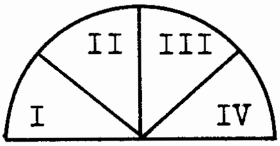
Table 6. Comparisons of frequencies of right and left responses for North- and South-oriented groups with those of East- and West-oriented groups.

not), gives $\underline{x}^2 = 5.30$, $p < .05$ (considered to be significant).

When all three of the preceding responses are present and in the same direction, subjects tend to repeat this rather than choose the alternative arm of the maze. Of 1581 responses, all preceded by three choices in the same direction, 883 followed this and 698 did not. When compared to an expected proportion of 0.5, this gives $\underline{x}^2 =$ 21.64, $p < .0001$.

Experiment II- Spontaneous Orientation Reactions

The results of a preliminary examination of the data are illustrated in Figure 5. The number of planarian paths recorded in each of four quadrants dividing the polar coordinate grid are shown for each orientation of the apparatus. Quadrants were designated as diagrammed here, with those paths on the lines (-45°, 0°, +45°, and +90°) assigned to the left quadrant.



Quadrant I	-90° to -45°
Quadrant II	-45° to 0°
Quadrant III	0° to $+45^\circ$
Quadrant IV	$+45^\circ$ to $+90^\circ$

Because of the asymmetrical light situation, the low frequency of paths in Quadrants III and IV (Figure 5) is expected. Concerning the responses toward the left half of the grid, the only observable difference seems to be between the east-oriented subjects and the other three

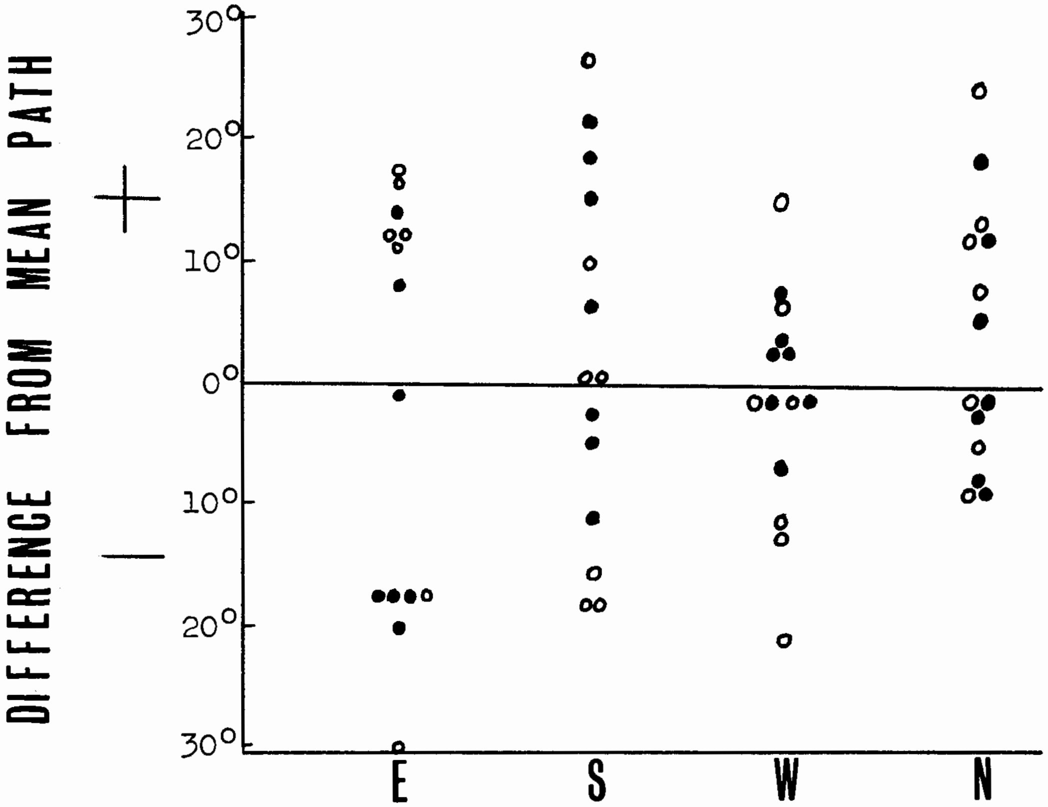


Figure 6. Difference between mean path in each observation period and the mean path for the corresponding period for all four directions taken together. (• AM observations; ○ PM observations)

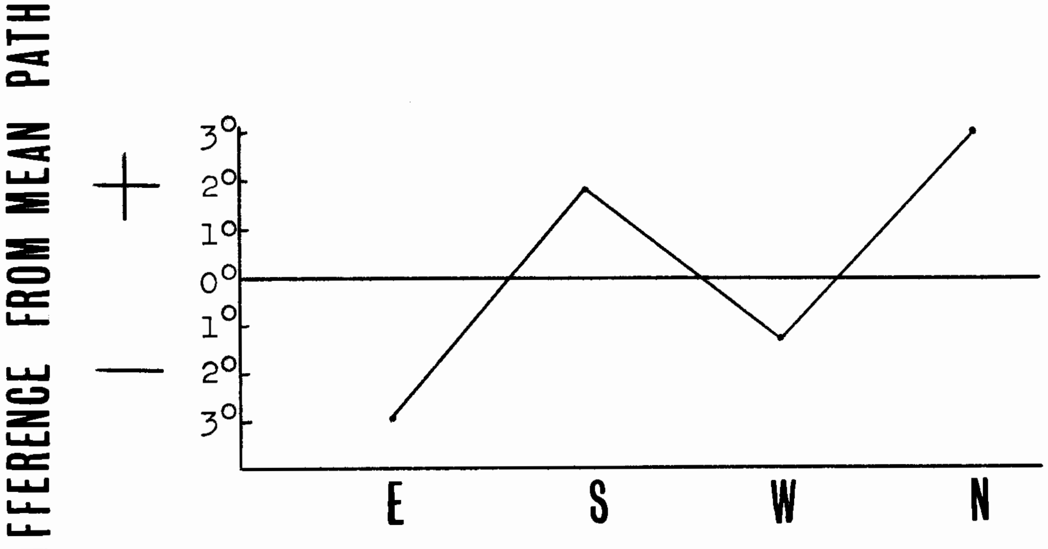


Figure 7. Mean path of all observation periods for each direction expressed as the difference from the mean path for all directions (-15.44°).

orientation groups. The former suggests greater left-turning (largest number of observations in Quadrant I), while the latter groups suggest greater right-turning (largest number of observations in Quadrant II). The small quantitative differences between these groups would not be significant in any test of validity and is cited only as a characteristic of the frequency polygons.

The mean paths for morning, evening, and combined daily observation periods are given in Table 5 for each orientation of the apparatus and also for all orientations combined. The closeness of mean paths and the large standard deviations make it impossible to see any noticeable differences between these groups.

When the average path for each of the fourteen observation periods (for each compass direction) was computed as the difference from the mean for the four directions of the corresponding period, the results in Figure 6 were obtained. For example, one entry represents the mean path of E-oriented subjects (on the first day, morning observation period) expressed as the difference from the combined mean path of E-, S-, W-, and N-oriented subjects on their respective (first day, morning) observation periods. A tendency for increased right-turning in the south- and north-oriented groups and left-turning in the east- and west-oriented groups is evident. This is shown more clearly in Figure 7 which expresses the mean path of all observation periods for each orientation as the difference from the mean path for all orientations combined. A definite ability of planarians to distinguish between N-S and E-W orientations is suggested here. In spite of this observed tendency for a greater amount of right-turning in the N-S groups than the E-W groups, application of a t test shows that this difference is not significant. Comparison of the mean path of all observations in the east- and west-oriented groups (-17.7°) with that of all of the observations in the north- and south-oriented groups (-13.1°) gives a t value of 0.96 (not significant).

Geomagnetic Effect on Maze Behavior

The observation of a slight tendency for increased right-turning in the south- and north-oriented groups over that of the east- and west-oriented groups (Figures 6 and 7) prompted an examination of a possible effect of this type in maze performance. If the earth's magnetic field has a similar effect on maze behavior, it would be expected that the right-reinforced subjects would exhibit better performances when the apparatus faced north or south than when it faced east or west. Likewise, the left-reinforced subjects may be expected to perform better when the apparatus faced east or west than when it faced north or south. It might also be predicted that control animals would choose the right arm of the maze more often in the north and south orientations of the apparatus and the left arm more often in the east and west orientations. The results of these comparisons are summarized in Table 6. The figures in Parts A and B of the table are the total number of correct responses for experimental subjects; the figures in Part C are the total number of right and left choices for control subjects. No attempt was made to obtain quantitative measures of differences here, because it can easily be seen that the groups being compared are very close in magnitude. The predictions mentioned above are not supported by the maze behavior in this study.

DISCUSSION

Instrumental Conditioning

Several factors must be considered before the results of this study can be accepted or rejected as evidence of instrumental learning in planarians. These will be discussed, however, no clear-cut statement to this effect can be advanced at this time, due to the excessive amount of disagreement in both the data cited here and the conclusions garnered from previous research.

If an attempt is made to establish a claim for instrumental learning, the experimental procedure must allow for the rejection of alternative interpretations for the increased proportion of correct responses on the second and third trial sets. The apparatus used here does rule out the sensitization effect, the main criticism of Ernhart and Sherrick' s (1959) instrumental learning paradigm. The latter included the use of light and darkness as cues. There were no cues offered in the present study, and the results of several t and chi square tests indicate that subjects exhibited no preference for one arm of the Y-maze over the other. It can be assumed that moisture and possible traces of stray light were present in equal amounts in both arms of the maze and provided no hint as to the choice which would lead to reinforcement.

If we consider only the type of data analysis used in previous studies of this type, there appears to be little justification for the view that learning has been demonstrated in the present study. Best and Rubinstein (1962) reported a significant ($p < .05$) preference for the reinforced alternative in Trial Set 2 when applying a t test to both the light-reinforced ($t = 3.6$) and dark-reinforced ($t = 3.1$) groups. A similar test in this study gives a t value of 0.25, not large enough to suggest this initial learning phase with any degree of validity. The present study also fails to duplicate the active rejection of the correct cue stimulus following the initial learning phase, as reported by Best and Rubinstein (1962) and Humphries and McConnell (1964). The variability in individual performance of the animals here is probably a major reason for the lack of agreement with these previous reports. A look at the large deviations from mean performances (Table 1), as well as several individual learning curves (Figure 4), will show how variability in individual performance can be masked by observing only group curves. The standard deviations obtained in this study are greater than twice the size of those reported by Best and Rubinstein (1962) in all trial sets.

The results of the present study and previous research suggest that the training regimen one imposes upon planarians is the most critical of all variables in accounting for the subsequent performance of the animals. Planarians will show "learning" or will fail to evidence "learned behavior" depending upon (1) the type of reinforcement used, (2) the amount of handling the animals are given, (3) the number of trials per day and the number of training sessions per week imposed upon the animals, (4) the species of planarian used, (5) the intensity of the noxious stimulus, (6) the cues in the maze allowing the animals to make a correct choice, and, possibly, (7) when and how often the animals are fed. In general, the early research indicated that less handling of the animals, imposing fewer trials per day and trial sets per week, using a relatively intense noxious stimulus, and feeding after training rather than before, result in a more stable demonstration of learning. Humphries and McConnell (1964) report that planarians learn better when avoiding the onset of a highly noxious stimulus (such as electrical shock) rather than when the reinforcement consists of the cessation of a continuous unpleasant situation (being returned to the home bowl from the confines of a maze).

A definite answer pertaining to the question of whether instrumental learning has been demonstrated in this study cannot be put forth at this time. The application of _t_ tests (see above) to the present data lead us to believe that no "learning" has been demonstrated, while the application of sign tests indicate opposite findings. Sign tests comparing maze performances cannot be ignored if we assume that some learning has occurred. A significant number of subjects exhibit a higher proportion of correct responses on Trial Sets 2 and 3 than on Trial Set 1, and also on Trial Set 3 than on Trial Set 2. If one accepts the validity of the sign test and defines learning as an increase in the probability that a correct choice will be made at a choice point, then it is clear that planarians can "learn" a maze such as the one used in this study. Because of the lack of agreement between the two tests (t test and sign test) applied to the data here, it is impossible to make any definite statement concerning the demonstration of instrumental learning in this study.

The failure of the present study to demonstrate unequivocal evidence of learning could be due to several aspects of the training procedure. First, the punishment (period in bright light) and reinforcement (return to home bowl) may not have been dissimilar enough to allow the organisms to easily discriminate between them. Despite the fact that experimental animals refused to run the maze approximately ten times as often as control subjects (200 "0" responses for R- and L-reinforced subjects; 20 "0" responses for controls), it could very well be that the bright light used as punishment here was not a highly noxious stimulus. Secondly, Humphries and McConnell (1964) report that Dugesia tigrina (the species used in this study) are typically less vigorous in the maze than Dugesia dorotocephala. Thirdly, the results indicating a possible tendency of planarians to follow previous paths suggest that washing the maze between each trial would be a more satisfactory procedure than merely allowing worms to crawl in it prior to the training period. Washing the maze between trials is the only way to completely eliminate any following behavior however slight it may be.

There seems to be a general consensus among researchers in this field that an adequate experiment cannot be designed without full knowledge of the general physiology and behavior of planarians. We especially need to conduct extensive observations of these worms under natural conditions. Evidence suggests that planarians are capable of a very high degree of exploratory learning; however, it is still debatable whether or not they demonstrate associative learning. They may be able to relate responses with one another and perhaps a very restricted group of stimuli and yet be unable to perform in any of the standard experimental situations designed to demonstrate associative learning.

Following Previous Paths

Even though chi square tests indicate the presence of a significant degree of path-following, this tendency seems only slight, at best, when considering the numbers involved. The fact that tests indicate a following of the immediately previous response and the self-response, but not the penultimate response, seems to be contradictory in itself. If we consider a hypothetical example (subjects A, B, and C, introduced into the maze in that order), it is reasonable to assume that if B follows A (the previous subject) a significant number of times, and C follows B (the previous subject) a significant number of times, then C should also follow A (the penultimate subject) with an equally high degree of significance. It could well be that there is no greater tendency for subjects to follow the immediately preceding response than the penultimate response. The smaller sample size used in testing the effect of the latter may have been a factor accounting for the apparent differences between the two comparisons in the chi square tests. Superficially, the values used in both tests do not appear to differ significantly from a 1:1 ratio (3483 follow previous, 3171 do not; 2736 follow penultimate, 2800 do not).

The effect of following the self-response can probably be accepted as real here, due to the highly significant results obtained in the chi square test ($\underline{x}^2 = 46.98$, $p < .0001$). It is possible that subjects could tend to continuously repeat their own responses from previous trials, without this having an effect upon the chance following of previous or penultimate subjects. This conclusion suggests an ability of planarians to sense and follow their own slime trails rather than fresher ones left by other worms.

The results of this portion of the present study emphasize the need for future, well-controlled experiments investigating the effect of path-following in planarians. The fact that both experimental and control animals were included in the data here could justify a rejection of the test results. It is impossible to predict whether the worms were more strongly influenced by slime trails or by the punishment and reinforcement of the experimental situation. The conclusion of past investigators (Best and Rubinstein, 1962; Humphries and McConnell, 1964), that contamination of maze pathways with mucous trails prior to training eliminates all chances of response bias, is indeed questionable. Again, the lack of knowledge of the planarian sensory apparatus prevents the serious acceptance of any present theory.

Spontaneous Orientation Reactions

These results confirm earlier reported ones (Brown, 1962a, 1962b; Barnwell and Brown, 1964) in demonstrating a compass-direction effect in planarians. A tendency for increased right-turning in the north- and south-oriented groups and increased left-turning in east- and west-oriented groups is evident. When the mean path of each direction is expressed as the difference from the mean for all directions, the results here are strikingly similar to those reported previously, however, the path deviations in this study are greater. The apparatus used in the present study is as close a duplication of that used by Brown as possible. Perhaps the larger path deviations here are caused by the use of a different species of planarian (Brown used _D. dorotocephala_), conducting studies in a different locality, or the assaying of a smaller number of planarian paths.

During this 28-day study, the moon phases present during each orientation of the apparatus were: East-new moon to first quarter; South-first quarter to full moon; West-centered on full moon; and North-last quarter to new moon. No attempt was made here to relate the effect of lunar rhythms on orientational responses because the apparatus faced a different compass direction during each phase of the moon. It would be impossible to separate lunar effect from compass-direction effect due to the lack of data from simultaneous testing of all orientations of the apparatus.

There remains no reasonable doubt that planarians are extraordinarily sensitive to very weak magnetic fields. Recent research investigating the influence of biological clocks and compass mechanisms on geographic orientation has been fruitful, however, its analysis is compounded by discoveries indicating the possible effects of other closely-related geophysical factors (electrostatic fields, barometric pressure, etc.). Orientational behavior patterns of animals seem to depend upon an input of information from the total geophysical scene, information which is integrated and then interpreted by the adaptively responding organism.

Geomagnetic Effect on Maze Behavior

The compass-direction effect observed in the spontaneous orientation reactions in this study had no influence on maze behavior. Three comparisons of the frequencies of right and left responses for the north- and south-oriented groups with those of the east- and west-oriented groups show no significant difference in any instance. Despite these results, the possibility that the geomagnetic field could influence an organism's response at the choice point in a maze cannot be ignored. There seems to be no definite reason why a geophysical factor (such as geomagnetism) could have a pronounced effect upon spontaneous orientation reactions and not upon maze orientations where a choice of paths is involved. Perhaps this magnetic effect could have been discernible here if a training procedure resulting in a more stable demonstration of learning had been used. If it can eventually be demonstrated beyond any reasonable doubt that the geomagnetic effect has no influence on maze learning behavior, at least two possible reasons can be suggested as to why this may be true. First, the worm may not be able to sense the change in direction of the magnetic field until after it has started to enter an arm of the maze at the choice point. Once the choice has been made, it is then too late for an adaptation, and the subject will continue in the chosen arm of the maze. Secondly, the maze situation may be so confining and aversive to the worms that their behavioral response to such a subtle geophysical factor may be completely eliminated.

Future studies of the possible effect of magnetism on maze behavior would be extremely valuable in helping to analyze past instrumental learning experiments, as well as adding information concerning the sensory capacities of planarians in their response to the geomagnetic field.

SUMMARY

Planarians were instrumentally conditioned in a simple two-choice Y-maze, with return to home bowl as the reinforcement, and bright light as the noxious stimulus. Some experimental subjects were trained to choose the right arm of the maze, some were trained to choose the left arm, and others served as controls. To investigate possible effects of the geomagnetic field on learning, subjects were conditioned in four groups, each with the apparatus facing a different compass direction. No differences between the four groups were observed. The results of sign tests indicate an initial learning phase (higher proportion of correct responses on Trial Sets 2 and 3 than on Trial Set 1, and also on Trial Set 3 than on Trial Set 2). The results of _t_ tests, on the other hand, do not support the view that "learning" has been demonstrated. There was no significant phase of active rejection of the reinforced alternative as reported in previous studies. A great variability in individual performances was demonstrated here. Previous research, as well as the results of this study, suggest that the training regimen one imposes upon planarians is the most critical of all variables in accounting for the subsequent performance of the animals.

Chi square tests indicate a slight tendency for these flatworms to follow previous paths in the Y-maze.

There seems to be a greater tendency for a worm to sense and follow its own mucous trail from the previous trial, rather than a fresher path left by another worm.

The effect of geomagnetism on the spontaneous orientation reactions of planarians was investigated in an experiment conducted concurrently with the instrumental conditioning study. Paths were assayed as the worms crossed a polar grid in an asymmetrically-lighted field. Observations were made with the apparatus facing each of the four compass directions. The results here confirm earlier studies in demonstrating a compass-direction effect in planarians. When the mean path of each direction is expressed as the difference from the mean for all four directions, increased right-turning in the N- and S-oriented groups, and increased left-turning in the E- and W-oriented groups is demonstrated. Path deviations in this study were larger than those reported previously.

The compass-direction effect observed in the spontaneous orientation reactions had no influence on maze learning behavior in the present study. Despite these results, the possibility that the geomagnetic field could influence an organism's response at the choice point in a maze should not be ignored. Suggestions are also offered as to possible reasons why maze behavior may not be affected by the earth's magnetic field.

The need for future studies to investigate the effects of path-following in planarians, and the effects of geomagnetism on the maze behavior of these organisms is stressed.

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APPENDIX

APPENDIX 1

Orientation ____________ Day ________

	Trial														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
R_1															
L_1															
C_1															
R_2															
L_2															
C_2															
R_3															
L_3															
C_3															
R_4															
L_4															
C_4															
R_5															
L_5															
C_5															

APPENDIX 2

Polar Grid

Orientation ____________

	Day														
Worm	Trial	1	2	1	2	1	2	1	2	1	2	1	2	1	2
1															
2															
3															
4															
5															
6															
7															